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SHORT COMMUNICATION

Courtship and mating behavior of *Grammostola schulzei* (Schmidt 1994) (Araneae, Theraphosidae), a burrowing tarantula from Argentina

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Abstract. *Grammostola schulzei* (Schmidt 1994) comprises a medium-bodied tarantula. Recently the species was found in Sierra de la Ventana, Buenos Aires, Argentina, where it lives in burrows always made under stones in rocky hills. Our objective was to offer a detailed description of courtship and mating, heretofore unknown, and to determine, under laboratory conditions, where copulation takes place: in the open field as proposed for all species of *Grammostola*, or at the entrance of the burrow. We carried out two series of experiments, completing 40 trials (20 in open arenas and 20 in burrowing arenas). All males initiated sexual behavior after contact with female silk. Courtship involved palpation, palpal drumming, body vibrations, and leg tapping. Palpation and palpal drumming appeared to operate in different ways. Females displayed typical behavior of a burrowing tarantula, making rapid bouts of tapping vigorously with the first pair of legs and palps. The mating position observed was typical of mygalomorphs, in which the female raises her body up and opens her fangs, the male uses the tibial apophyses to clasp the female's fangs, and finally begins a series of attempts at palpal insertion. The smooth slapping during clasping may serve to keep the female passive and relaxed. The dorsal flexion observed during mating and the number of successful matings in open arenas suggests that copulation would take place outside the burrow. We observed sexual cannibalism during the interactions but in these cases, we registered no previous courtships by the males.

Keywords: Theraphosinae, sexual display, copulation, female response

Resumen. *Grammostola schulzei* (Schmidt 1994) es una tarántula de tamaño medio. Recientemente la especie se colectó en Sierra de la Ventana, Buenos Aires, Argentina, donde vive en cuevas hechas siempre bajo piedras en cerros rocosos. Nuestro objetivo fue ofrecer una descripción detallada del cortejo y apareamiento y poner a prueba bajo condiciones de laboratorio dónde tiene lugar la cópula. Se realizaron dos series de experimentos completando 40 en total (20 en arena abierta y 20 en arena con cueva). Todos los machos iniciaron el comportamiento sexual luego de contactar con la seda de la hembra. El cortejo involucró movimientos de palpar, golpeteos de palpos, vibraciones corporales y golpes con el primer par de patas. Las secuencias de palpar y golpeteos de palpos parecen actuar de maneras distintas. Las hembras mostraron un comportamiento típico de tarántulas que habitan cuevas, realizando golpeteos rápidos con el primer par de patas y palpos. La posición de cópula observada fue similar a la de otras migalomorfas, en la cual, la hembra eleva su cuerpo y abre los quelíceros, el macho utiliza sus apófisis tibiales para engancharlos y por último comenzar una serie de inserciones palpales. Los golpes suaves durante el enganche servirían para mantener una condición pasiva y relajada de la hembra. La flexión dorsal observada durante la cópula y el número de cópulas exitosas en arena abierta sugiere que el apareamiento tendría lugar fuera de la cueva. Hemos observado canibalismo sexual durante las interacciones, pero en estos casos no hubo cortejo previo de los machos.

Few studies have been conducted on the reproductive behavior of tarantulas in general (Baerg 1958; Minch 1979), and the small amount of published literature that contains detailed behavioral descriptions (Shillington & Verrel 1997; Jackson & Pollard 1990) suggests that the traditional portrayal of mygalomorph display behavior as "simple" (Platnick 1971; Foelix 1996) is misleading. Moreover, an understanding of tarantula reproduction is necessary to facilitate captive breeding and to reduce pressures on wild populations caused mainly by human impact and pet commerce (Costa & Pérez-Miles 2002). The South American genus *Grammostola* Simon 1892 (Bücherl 1951), includes large-sized species whose carapace length varies from 20–30 mm (Postiglioni & Costa 2006). The great density and diversity of specimens of *Grammostola* spp. in Argentina has led researchers to think that Argentina is the dispersion center of the genus. In Argentina, it has a different biotope than Brazilian species (Schiapelli & Gerschman 1960). Bücherl (1951) described the reproductive biology of several species of *Grammostola* but only for medical or systematic purposes. Recently, two allopatric species living in Uruguay have received a considerable amount of attention regarding their reproductive biology: *Grammostola iheringi* (Keyserling 1891), which has been reported only from neighboring areas of Quebrada de los Cuervos, and *G. mollicoma* (Ausserer 1875) which is widely distributed throughout the country (Pérez-Miles & Costa 1992; Costa & Pérez-Miles 2002; Postiglioni & Costa 2006).

Grammostola schulzei (Schmidt 1994), is a burrowing species from Argentina whose males are much smaller than females (Males are 30 mm long) (Schmidt 2005). Bertani & Fukushima (2004) identified specimens from Cordillera de Los Andes (Argentina), Tandil (Buenos Aires, Argentina), and the species was also collected in Sierra de la Ventana (Buenos Aires, Argentina), where it lives in burrows made under stones in rocky hills (Ferretti & Ferrero 2006).

In this paper we offer a detailed description of courtship and mating under laboratory conditions. We provided two different types of arenas for the matings to occur: an open area similar to an open field as proposed by Costa & Pérez-Miles (2002) for all species of *Grammostola*, or at the entrance of the burrow.

METHODS

Spiders.—Five males and eight females of *G. schulzei* were collected by hand during the summer of 2006 in the locality of Sierra de la Ventana, Buenos Aires, Argentina (38°07′63″S, 61°47′30″W); and the observations were made during the November–December period of 2006. Due to cannibalism, we needed to capture three more males to maintain the number of five individuals to obtain the data. Voueher specimens were deposited in the Entomological Collection of Zoology of Invertebrates II, Universidad Nacional Del Sur, Buenos Aires, Argentina. All the females molted in the laboratory between January and February of 2006 so they had no stored sperm. Individuals were raised in glass vials of 13 cm diameter or glass rectangular cages of 30 × 14 cm according to their size. They had a substrate of soil, water provision, and were fed *ad libitum* with coekroaches (*Blattela germaniea*) and *Tenebrio* sp. larvae (Coleoptera). The average environmental temperature was 26.7° ± 1.52° C.

We carried out two series of pairings in cages measuring 30 × 35 and 30 cm high and completed 40 trials (20 each). The first series was performed in conditions with a layer of soil of approximately 2 cm deep, which we called an "*open arena.*" In the second series, the layer of soil was 10 cm deep and a burrow was excavated against the glass wall allowing for observation. This constituted the "*burrowing arena.*" This burrow simulates natural conditions and was recommended for experiments (Postiglioni & Costa 2006). In both situations, the female was placed in the eage at least 24 h before trials to permit pheromone deposition with silk on the soil surface. The individuals in a pair were never tested together more than once and none was used in more than one test on a given day. Each pair of spiders was reused at least 1 day after the first experiment, but in different combinations. Individuals were randomly assigned to pairs and experimental series.

The observations began by carefully depositing the male far from the burrow entrance (*burrowing arena*) or the female's position (*open arena*) and ended after a 60 min period of non activity or when the copulation concluded.

Description and analysis.—Encounters were recorded by direct observations, notes, and videotape (85% of encounters) with a Sony Cyber-Shot DSC-H2 (60 fps). Video records were analyzed using the PC program Video edit magic 4.23 in order to accurately describe behavioral patterns, using slow motion and single frame advance modes. Normality and homogeneity of variance of continuous variables (durations of the behaviors) were tested using the Kolgomorov-Smirnov and Levene tests respectively. Parametric Student's *t*-test (non-paired samples) was used for parametric durations. Non-parametric Mann-Whitney *U*-test (non-paired) for frequencies and Kolgomorov-Smirnov two sample tests were used for non- parametric durations. All statistical analyses were performed using SPSS version 14.0 for Windows (2005). Averages are given ± 1 standard deviation.

RESULTS

We recorded a total of 13 successful matings during 40 trials, 9 in the *open arena* and only 4 in the *burrowing arena*. All courtship behaviors were initiated by males after contact with female silk. In the unsuccessful courtships (27 in all encounters) where the copulation failed, all involved unreceptive females either did not offer any signal or attacked the males.

Courtship behavior.—Duration of courtship was of 18.98 ± 13.82 min, and in 77% of the cases courtship started with male "palpating," a behavior which consisted of the two palps moving up and down in an alternating manner, hitting the soil with a constant velocity. Bouts of palpating were highly variable in duration (8.15 ± 5.93 s, range = 1.15–21.26 s) and were always performed at > 20 cm from the female. "Palpal drumming" was also common and started at low velocity (4.5 ± 1.73 bouts/s), frequency of 3 Hz, and increased sequentially (12.5 ± 2.08 bouts/s), frequency of 13 Hz. The duration of a bout of palpal drumming was 6.27 ± 1.39 s and was more conspicuous after any movement by the female or near her (~ 10 cm). It often interrupted the bouts of palpation. When comparing mean durations of male signaling bouts (palpation and palpal drumming), no significant differences were found (non-paired *t*-test, $t = 1.69$, $P > 0.05$). In all cases with palpal drumming, the male displayed body vibrations caused by the movements of legs III and could not be quantitatively described using the video because it was not possible to observe male movements in detail. Leg tapping involved only legs I and consisted in elevating a leg, extending it, and lowering it rapidly to hit the soil. The pattern could include each leg independently or simultaneously and was always observed in conjunction with palpating. During courtship, the sequence of palpation, palpal drumming, vibration, leg tapping was more frequent. The frequency of these bouts in *open* and *burrowing arenas* are given in Table 1. We observed males laying down silk over the female's silk strands in 21 interactions (3.09 ± 2.58 bouts/interaction) during courtships.

One female (*open arena*) made rapid bouts of tapping vigorously at low amplitude with the first pair of legs and palps by flexing, lifting and lowering against the substrate. This was carried out seven times after the third palpal drumming by the male and he reoriented towards the female. No statistical differences were found using the Mann-Whitney test either when comparing the frequency of bouts of palpation in *open arena* and *burrowing arena* ($U = 141.5$, $P > 0.05$), or when comparing the frequency of bouts of palpal drumming in *open arena* and *burrowing arena* ($U = 160.5$, $P > 0.05$).

Copulation.—For mating, the male started to display spasmodic "beats" that involved only the second pair of legs that vigorously hit the body of the female during clasping and unclasping. The pattern for this could be alternate or synchronous. We observed a total of 226 bouts (16.14 ± 10.20 bouts per copulation). The female performed "gaping display" in which she raised her carapace, extended the two first pairs of legs, and also opened her fangs. Typically, when the female did this behavior, the male lunged toward the female and clasped her chelicerae with the tibial apophyses. After this, the male pushed up the female's body, raising her to reach a position that formed an angle of 60–80° between carapace and abdomen and the female entered a passive state, becoming quiescent. During clasping, the male sometimes displayed smooth slapping over the female's carapace by the metatarsus and tarsus of legs II. This was in either alternate or synchronous form.

Finally the male began a series of palpal insertion attempts. The number of insertions per copulation was 4.23 ± 3.70 SD. The

Table 1.—Behavioral units performed by five males of *Grammostola schulzei* during courtship in *open arena* and *burrowing arena*. *n* = total number of courtships where the behavioral unit took place; the mean is the number of bouts per courtship of all males.

	Open arena			*Burrowing arena*		
Behavioral units	Mean	SD	*n*	Mean	SD	*n*
Palpate	13.46	13.27	15	17.22	21.93	9
Body vibrations	6.91	8.10	12	9.87	7.88	8
Palpal drumming	10.25	14.06	16	17.40	19.72	10
Leg tapping (one leg)	18.28	42.59	14	27.00	15.72	4

duration of copulation was 204.52 ± 183.13 s. When a female was inside the burrow, we observed two copulations at the burrow entrance and two outside the burrow at a distance of approximately 10 cm. No statistical difference was found when comparing duration of copulation between *open arena* and *burrowing arena* using the Kolmogorov-Smirnov test (non-paired, $Z = 1.10$, P 0.05). We recorded three cases of sexual cannibalism in which the female attacked and killed the male before mating, one case in *open arena* and two in *burrowing arena*. Also we observed two attacks by females against males before mating, but they were able to escape with a series of injuries.

DISCUSSION

Courtship behavior.—Early work on mygalomorphs proposed that mygalomorphs lacked chemical cues in sexual communication (Baerg 1958; Platnick 1971); however, our results agree with more recent studies that confirm the existence of female contact sex pheromones (Minch 1979; Prentice 1997; Shillington & Verrell 1997; Yañez et al. 1999; Costa & Pérez-Miles 2002). Palpation occurs at a greater distance than palpal drumming and generally initiates the courtship; palpal drumming may serve as a short-distance communication mechanism near the burrow entrance (Pérez-Miles et al. 2007). These behaviors were considered the same behaviors that, in other studies, were referred to as palpal drumming (Costa & Pérez-Miles 1992; Shillington & Verrell 1997; Yañez et al. 1999; Quirici & Costa 2005), but in *G. schulzei* they seem to operate in different ways because, after a response from the female, the male only engages in palpal drumming and stops palpating. Although we found no differences in duration of these bouts, the frequency (Hz) and velocity are different. Palpation could be similar to the behavior described for other mygalomorphs (Jackson & Pollard 1990). Body vibration is a widespread behavior in the courtship of Theraphosidae (Minch 1979; Prentice 1997; Costa & Pérez-Miles 2002) and is very common in *Acanthoscurria suina* Pocock 1903 and *Enpalaestrus weijenberghi* (Thorell 1894), two burrowing tarantulas (Quirici & Costa 2005; Pérez-Miles et al. 2007). In *G. schulzei* body vibration always occurs in synchrony with palpal drumming and this is not the case for other species of *Grammostola* (Pérez-Miles & Costa 1992; Postiglioni & Costa 2006). Leg tapping had been described by Baerg (1958), Prentice (1997) and Shillington & Verrell (1997) for *Aphonopelma* spp. males and for *G. mollicoma* and *G. iheringi* by Postiglioni & Costa (2006), indicating that this behavior could be acting as a series of communicatory signals in sexual context, maybe using a seismic and/or acoustic channel. We observed leg tapping far away from the female's position acting as primary signals; moreover, this species displays leg tapping only with the first pair of legs.

The laying down of silk by males was also reported for *Brachypelma klassi* (Schmidt & Krause 1994) (Yañez et al. 1999) and these authors suggest that it may be a method of interfering with chemical or tactile cues that may be used by subsequent males to locate the female. However, our results showed that the males had no trouble finding the female's position and mate successfully.

The leg tapping displayed by the female had not been observed in other species of *Grammostola* and is described here for the first time. Coyle (1986) observed a similar behavior in a Dipluridae. In Theraphosidae, it was first observed by Prentice (1992) for three species of *Aphonopelma*. Quirici & Costa (2005) found that both *E. weijenberghi* and *A. suina* respond to male courtship from inside their burrows suggesting that such action would not only inform the male about her willingness to copulate, but also help the male orient himself towards the burrow entrance.

Curiously, this behavior was performed by one female in *open arena* and the male reoriented himself towards the female, suggesting that this behavior, at least in *G. schulzei*, seems to be important for orientation rather than indicating the receptive state of the female because we obtained more successful matings with females that did not respond. Moreover, the male who elicited this response from a female had an unusual additional thorn spine on the internal tibial apophyses, and this deformity could have generated novel signals maybe in leg tapping as was observed for body vibrations in *E. weijenberghi* (Pérez-Miles et al. 2007).

Copulation.—The male's spasmodic beating with legs II is unique to *Grammostola* and could be a synapomorphy for this genus (Costa & Pérez-Miles 2002). These authors also suggest that its function could be to relax the female's fangs, taking into account that it is mainly displayed during clasping and unclasping. The mating position observed in the present study was typical of mygalomorphs (Coyle & OShields 1990). The dorsal flexion observed implies that the copulation would take place outside the burrow. Moreover, we obtained more successful matings in the open field than at the burrow entrance without any problem of equilibrium, but the low number of successful matings in the *burrowing arena* could be attributed to the absence of the female's silk in some interactions. This raises the question of why the female shows a characteristic behavior of a burrowing tarantula that "pledges" the species to strictly copulate at the burrow entrance. This mating system was proposed for the burrowing tarantulas (Costa & Pérez-Miles 1992; Shillington & Verrell 1997; Yañez et al. 1999; Pérez-Miles et al. 2007) and seems to be inconsistent with our results, but this needs to be elucidated with more data.

The smooth slapping was not observed for other theraphosids and is reported here for this species. This behavior may serve the same function as palpal boxing in *Brachypelma klassi*: to maintain the female's "passive" state (Yañez et al. 1999).

The number of insertions was similar to that reported for other species of Theraphosidae (Baerg 1958; Minch 1979; Pérez-Miles & Costa 1992) but the copulation duration was brief (lasting only a few minutes) in relation to other species of *Grammostola* (Pérez-Miles & Costa 1992). This could minimize the predation risk of the couple in an exposed mating site (Punzo & Henderson 1999). We observed sexual cannibalism, but its absence seems to be the rule for theraphosids (Costa & Pérez-Miles 2002). However, even a low frequency of cannibalism may be a significant problem for males, a problem solved by courtship (Jackson & Pollard 1990). In fact, in all the cases in which the females attacked and killed or injured males, we registered no previous courtships by the males.

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